

# THE ANTIMICROBIAL ACTIVITY OF SOME EXTRACTS OF FERN GAMETOPHYTES

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## Abstract

The nature freely offers us many resources for health and beauty. The ferns and their therapeutic properties are less exploit in Romania, except *Lycopodium clavatum* and *Equisetum arvense*. Some of the fern properties were demonstrated, like antioxidant, antimicrobial, antiviral, antihelminthic properties. Plants are reasonable alternative to synthetic drugs, avoid the side effect and high cost of synthetic drugs production. Also, the drug resistance bacteria can be controlled using plant derived remedies.

In this study the antimicrobial effect of methanolic and ethanolic extracts from three fern species were tested. The extracts were gained from gametophytic stage of ferns obtained *in vitro*. The most obvious effect was observed for *Asplenium trichomanes-ramosum* extract. The total polyphenols and flavonoids content were established, too.

Keywords: fern, alcoholic extracts, gametophytes.

## 1. INTRODUCTION

Plants have been used in traditional medicine since ancient times to cure the diseases, so the pharmacological effects of some medicinal plants and their interactions with human body are already known and certified (Nascimento et al., 2007). Plants provide a source of active compounds, beneficial as alternatives to synthetic ones, in addition to side effects of drugs, high costs of synthetic drug production and antibiotic resistance of microorganisms (Hussain et al., 2011). An impressive number of modern drugs derive from natural sources to avoid side effects and the most of them is based on traditional medicine agents (Doughari et al., 2008). Medicinal plants are an important source of natural antimicrobial agents and antioxidants (Khaing, 2011). The understanding of chemical principles of herbal drugs is still insufficient, because the limitation of their wide-spread, especially in Western societies (Zhu et al., 2004).

Among all active ingredients from chemical composition of medicinal plants, only some of them have therapeutic effects and are important for medical world. Ferns are not considered traditional medicinal plants, but some active ingredients of them are recognized as substances with significant effects against pathogenic agents.

The aim of this study was testing the antimicrobial potential of some regional fern extracts in gametophytic phase obtained *in vitro*. The polyphenols and flavonoids content were established, too.

## 2. MATERIAL AND METHOD

In this study the fern gametophytes obtained *in vitro* were used. Ferns were collected from natural sites and gametophytes were obtained in growth chamber for species: *Asplenium trichomanes* (1), *Polypodium vulgare* (2), *Asplenium trichomanes-ramosum* (3).

### Extracts preparation

The methanolic (A) and ethanolic (B) extracts were obtained. The extracts were prepared at room temperature in 24 hours by adding 50 ml methanol (for methanolic extracts) or ethanol (for ethanolic extracts) over 5gr of squeezed gametophytic material (for each fern species).

The raw extracts resulted from it were stirred and then filtered through 8 - layered muslin. The filtrates were spine out at 6000 rpm for 10 minutes at room temperature. The supernatants were filtered by Whatman No. 1 filter paper (Parihar et al., 2006).

#### **Polyphenols content**

The total polyphenols content was determined according to the method of Singleton and Rosi (1965) with Folin-Ciocalteu reagent. Gallic acid was employed as calibration standard; the results were expressed as mg Gallic acid equivalents (GAE) per g FW/ml raw extract.

#### **Flavonoids content**

Measurement of flaonoids content was performed according to Zhishen method (1999), the results were expressed as catechin equivalents (CE/100g FW) in 100 g dry weight.

#### **Antimicrobial effect**

The antimicrobial activity of fern extracts was tested by disc diffusion method, on 5 bacterial strains: *Staphylococcus aureus* ATCC 25923 (Sa); *Streptococcus sp.* (St) and *Escherichia coli* (Ec) - strains isolated from human microbiota; strains 23S (B 23S) and 21F (B 21F) isolated from soil. Standard antibiotic discs (Ampicillin 10µg, AMP) were used as a positive control; the negative control was the solvent, methanol (M), respectively ethanol (E).

An overnight culture of each bacterial strain was obtained at 37°C by inoculating the microorganisms into 2 ml nutrient broth. The bacterial cell suspensions were uniform inoculated on the surface of nutrient agar in plates. After inoculation, the discs were put on the surface and the plates were incubated at 37°C for 24 hours.

The antimicrobial effects of fern extracts were estimated by measuring the diameter of inhibition growth zone (in millimeter), as a clear, distinct zones of inhibition around discs, compared to positive and negative control.

### **3. RESULTS AND DISCUSSIONS**

In terms of polyphenols and flavonoids content, this study revealed some differences between fern species and between methanolic and ethanolic extracts of the same species, too. Hence the content of total polyphenols was higher for extracts of *Asplenium trichomanes* and *Polypodium vulgare* than the other species; ethanolic fern extracts presented a higher content of total polyphenols than methanolic ones, with one exception, *Asplenium trichomanes* (table 1), but the differences were small.

Concerning the content of flavonoids, the methanolic extract of *Polypodium vulgare* revealed the highest content, and then the ethanolic extracts of the same species and methanolic extract of *Asplenium trichomanes-ramosum* revealed high values. Methanolic fern extracts presented a higher content of flavonoids (table 1). Among all extracts, methanolic and ethanolic extracts of *Polypodium vulgare* denoted high values for both polyphenolic and flavonoids content.

*Table 1. Total polyphenols and flavonoids content*

<b>Sample</b>	<b>FLAVONOIDS</b> (CE/100g FW/ ml extract)	<b>TOTAL</b> <b>POLYPHENOLS</b> (mg GAE/ml raw extract)
1A	0.108	0.292
1B	0.098	0.236
2A	0.254	0.265
2B	0.196	0.281
3A	0.190	0.216
3B	0.118	0.217

The methanolic fern extract from gametophytic phase of *Asplenium trichomanes* was not efficient against tested bacterial strains, but the ethanolic extract inhibited the growth of *Streptococcus* sp., *Escherichia coli* and B 23S strain (from edafic microbiota). The inhibition growth zones were larger than the control (ethanol) one just with 1 mm.

The extracts of gametophytic phase of *Polypodium vulgare* were efficient against *Escherichia coli* (methanolic extract) and B 23S strain from edafic microbiota (ethanolic extract).

The most efficient extracts were extracts from *Asplenium trichomanes-ramosum* gametophyte. The inhibition growth zones induced by methanolic extract were obvious against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli*, while ethanolic extract was efficient against B 23S strain from edafic microbiota (table 2).

**Table 2. Antimicrobial effect of methanolic (A) and ethanolic (B) extracts**

Sample	Microorganism				
	Sa	St	Ec	B S23	B 21 F
	Inhibition zone (IZ) mm				
1A	0	0	0	0	0
1B	0	8	8	9	0
2A	0	0	7	0	0
2B	0	0	0	7	8
3A	9	6	8	0	0
3B	0	0	7	8	7
AMP	15	14	14	25	15
M	0	0	0	0	0
E	0	8	7	7	7

#### 4. CONCLUSIONS

This study denoted the content of total polyphenols was in general higher for ethanolic fern extracts than methanolic ones, with small differences. The highest polyphenolic content was determined for extracts of *Asplenium trichomanes* and *Polypodium vulgare*. Some differences between fern species and between methanolic and ethanolic extracts of the same species were observed, too.

Methanolic fern extracts presented a higher content of flavonoids. The highest content of flavonoids was determined for methanolic extract of *Polypodium vulgare*.

Among all extracts, methanolic and ethanolic extracts of *Polypodium vulgare* denoted high values for both polyphenolic and flavonoids content.

Among methanolic extracts, the most obvious antibacterial effect was obtained for extract of *Asplenium trichomanes-ramosum* gametophyte; the most manifest antibacterial effect of ethanolic extracts was in case of *Asplenium trichomanes* extract. Bacterial strain B 23S from edafic microbiota and *E. coli* strain were the most inhibited bacteria by tested extracts (especially ethanolic ones).

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